# EXECUTIVE SUMMARY

Plants are a great reservoir of various phytochemicals, exhibiting several therapeutic activities, paramount for drug development industries. Human civilization has exploited this reservoir since the Neolithic age for disease treatment and cure, preserved in traditional knowledge. However, the revolution in science and technology put forth the chemical synthesis of scientifically proven, pharmacologically active drugs in the market, due to which traditional medicines lagged. However, the uprisal of several side effects and low efficacy in synthetic drugs has again stirred the interest of global pharmaceutical companies in natural and herbal products. Worldwide, researchers are trying to rediscover their potential as the source of safer drug candidates. Though herbal medicines have been used since ancient times, their chemical characterization and therapeutic activity validation have not been scientifically proven. Hence, there is a requirement for research impetuous for the modernization of herbal medicines. Therefore, a new class of drugs has been defined to fill the lacuna created due to the lack of research in natural product treatment: "*Phytopharmaceutical drugs*", a standardised purified fraction derived from the medicinal plants or its part. The present thesis has been conducted on an Indian medicinal plant, *Aegle marmelos* (L.) Correa, for developing phytopharmaceutical ingredient(s) possessing active compounds against inflammation.

*Aegle marmelos* is known to exhibit several therapeutic activities. It is known to balance all three doshas and relieves pain, dyspepsia, gastritis, and abdominal colic pain. In addition, several studies claim that the plant possesses anti-diarrhoeal, antimicrobial, antiviral, anticancer, chemo-preventive, antipyretic, analgesic, anti-ulcerative, diuretic, antifertility, and anti-inflammatory properties. Furthermore, the plant contains several valuable phytocompounds such as marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegeline, umbelliferone, marmelin, fagarine, marmesin, luvangentin, and auroptene. Despite this, not a single *A. marmelos* formulation has been granted Certificate of Pharmaceutical Product (COPP) as per the WHO GMP Certification Scheme by the Central Drugs Standard Control Organization (CDSCO).

Despite its medicinal value, systematic studies on variations in primary and secondary metabolites of *A. marmelos*, are still lacking. Identification of bioactive marker

compounds in medicinal plants necessitates the exploration of the entire range of primary and secondary metabolites. **Chapter 4** mainly focused on pharmacognostic and phytochemical screening in various parts of *A. marmelos,* viz., leaf, fruit, twig, and bark, followed by the estimation of nutritional content, elemental, pesticide residues, and aflatoxin impurities. The study also addresses variations in primary and secondary metabolites in different parts of *A. marmelos* using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy for bioprospection. Additionally, for the evaluation of primary and secondary metabolites, this thesis contributes to the development of a robust high-performance thin-layer chromatography (HPTLC) method. This method enables the simultaneous determination of marmelosin, psoralen, and umbelliferone in various parts of *A. marmelos* as a prerequisite for quality maintenance.

**Chapters 5 and 6** focused on the enrichment of predominant/underexplored phytoconstituents present in the extract of *A. marmelos* fruit/leaves. Coumarins being the major phytoconstituents in the *A. marmelos* and also hold significance due to their diverse biological activities and potential applications were selected for enrichment. A total of five coumarins viz., marmelosin, marmesin, psoralen, scopoletin, and umbelliferone, were enriched, characterized, and quantified in the coumarins-enriched fraction. The enrichment process of the desired bioactive markers was designed to obtain maximum yield of the compound(s) and may have industrial applicability. Besides this, three well-established primary metabolites, quinic acid from *A. marmelos* leaves, and *myo*-inositol, and 2,4-di-*tert*-butyl phenol from *A. marmelos* fruits renowned for their nutritional and pharmacological significance were enriched. Detection and quantification were meticulously executed through advanced analytical techniques, encompassing HPTLC, high performance liquid chromatography (HPLC), GC-MS, direct sample analysis by mass spectrometry (DSA-MS), fourier-transform infrared spectroscopy (FTIR), and NMR. The bioactivity of the enriched fractions has been analyzed by performing various *in silico, in vitro* and *ex vivo* assays. *In silico* molecular docking of selected target metabolites was performed by targeting Lipoxygenase (LOX2) enzyme, for the assessment of anti-inflammatory activity. 5LOX-2 enzyme is known to be involved in pro-inflammatory activity, therefore chosen for the molecular docking. Apart from the *in silico* analysis, various *in-vitro* assays, such as anti-proteinase activity and lipid peroxidase assay, were checked, as a

confirmatory test. Furthermore, the probable adverse effects of the enriched fractions were checked by *in silico* Absorption Distribution Metabolism Excretion and Toxicity (ADMET), *in vitro* cell cytotoxicity, and scanning electron microscopy (SEM) study.

The coumarins-enriched fraction was further used for formulation development. A topical gel from the coumarins-enriched fraction was developed and its stability parameters were checked concerning industrial production which is elucidated in **Chapter 7**.

Overall, the data obtained from the present study may be used by the pharmaceutical industries and the scientific community for the quality production of a processed phytopharmaceutical drug formulation, which will pave the path forward for more advanced research in innovative medicines production for the society.